DIFFERENCES IN THE CLINICAL AND LABORATORY FEATURES OF ONCHOCERCIASIS IN ENDEMIC INDIVIDUALS AND TEMPORARY RESIDENTS

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ABSTRACT

Background. Many parasitic infections have different presenting features in endemic populations and immunologically naïve temporary residents. Prior studies describe a clinical syndrome characterized by dramatic acute symptoms and hypereosinophilia in travelers infected with the filarial parasite *Loa loa*, in contrast to a parasite-induced subclinical (asymptomatic) state in chronically infected endemic hosts. Few studies have examined the relationship between endemicity and disease manifestations in other filarial infections. We aim to directly compare the clinical characteristics and laboratory findings in endemic individuals (END) and temporary residents (TR) infected with the filarial parasite *Onchocerca volvulus*.

Methods. We identified all patients definitively diagnosed with active *Onchocerca volvulus* infection at the National Institutes of Health between 1976 and 2016. All study subjects received a detailed baseline assessment including a comprehensive history, physical examination, ophthalmologic evaluation, and extensive laboratory investigations including testing for parasitic coinfections. We performed additional parasite-specific serologic testing on stored patient sera.

Results. Forty temporary residents (TR) and 36 endemic subjects (END) had active onchocerciasis. All END patients were symptomatic, whereas 12.5% of TR reported no *O. volvulus* symptoms ($P = .06$). Papular dermatitis was more common in TR (47.5% vs 2.7%, $P < .001$), while pigmentation changes were more often observed in END (41.7% vs 15%, $P = .01$). Only END patients reported visual disturbance (13% vs 0%, $P = .03$).

Onchocercal eye disease was detected in 22.6% of END patients and in only 1 TR (3.3%, $P = .053$). There was no difference in baseline eosinophil levels between groups ($P = .5$), and one third of subjects in both groups had a normal eosinophil count. END patients had higher filarial-specific IgG4 levels and were more likely to be positive for IgG4 antibodies to OV16.

Conclusions. Although there is substantial overlap in the presentation of *O. volvulus* infection in TR and END populations, skin manifestations differed, and ocular involvement was almost exclusively observed in END patients. Unlike *Loa loa*, differences in clinical presentation do not appear to be eosinophil-mediated, and instead may reflect other factors such as chronicity of infection.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

General background

Onchocerciasis is a neglected tropical disease caused by the filarial parasite *Onchocerca volvulus*, that can lead to chronic debilitating skin and eye pathology. At least 18 million people are infected, and 180 million live in at-risk areas worldwide.[1,2] Also known as "river blindness", onchocerciasis is the second leading infectious cause of blindness, and is responsible for 270,000 cases of blindness and 500,000 cases of visual impairment worldwide.[3–5]

Chronic skin and eye disease caused by *O. volvulus* has many devastating long-term socioeconomic consequences. Visual impairment due to onchocerciasis historically occurred in up to 40% of adults in hyperendemic areas, ultimately driving population migration to lower transmission zones and away from arable land.[5] In comparison to healthy individuals, those affected by onchocercal dermatitis or visual impairment have substantially lower economic productivity examining a variety of measures, including income, ability to maintain employment, length of workday, and ability to concentrate at work.[6] In 2015, onchocerciasis resulted in the loss of an estimated 1.1 million disability adjusted life years (DALYs), an age-standardized DALY rate of 15.5 per 100,000.[7] Current estimates likely underrepresent the true disease burden and population impact, due to sparse epidemiologic data in the remote areas of sub-Saharan Africa where the disease is most prevalent.[8–10]

Onchocerciasis is currently endemic in 31 countries in Africa, which harbours over 95% of global disease.[1] Limited foci of active transmission still exist in Yemen, Venezuela and Brazil, in the wake of successful elimination campaigns in previously

endemic Latin American countries including Colombia, Ecuador, Mexico, and most recently Guatemala as of July, 2016.[1,11] Onchocerciasis control and elimination programs have dramatically reduced global disease burden through mass drug administration with ivermectin, and aerial insecticide spraying. The disease almost exclusively affects rural populations living in poverty, as the *Simulium* blackfly vector reproduces near remote fast-flowing rivers and streams. Infected blackflies have a limited flight range and typically bite humans while accessing fresh water for washing, bathing, and agricultural or occupational use.[12]

Humans are the only host for *O. volvulus.* *Simulium* blackflies inoculate third-stage (L3) larvae into human skin, where they penetrate deeper subcutaneous tissues and mature into adult worms over 6-12 months. Adults coalesce into fibrous nodules termed "onchocercomata", and each adult female typically releases 1,000-3,000 immature larvae (microfilariae) each day.[4] Adult worms will ultimately produce millions of microfilariae over the course of their 9-15 year lifespan.[4,9] Microfilariae migrate throughout the skin, eyes, and lymphatics, and survive for 6 to 24 months.[4,5] Blackflies, in turn, ingest dermal microfilariae, which then mature into L3 larvae in the arthropod vector as *O. volvulus* completes its lifecycle. The commensal intracellular bacteria *Wolbachia* can be found in all life cycle stages and plays an essential role in embryogenesis and parasite survival.[4,13]

O. volvulus causes human disease by inducing an inflammatory immune response to dying microfilariae in host tissues, leading to tissue damage as a byproduct.[5] Live migrating microfilariae do not appear to provoke a significant inflammatory response, but rather it is the release of antigen from both dying microfilariae and endosymbiotic

Wolbachia that leads to immune activation and inflammation.[4,13–15] An extreme illustration of this is the Mazzotti reaction, in which treatment with diethylcarbamazine rapidly kills microfilariae, and in doing so provokes potentially life-threating acute hypotension, tachycardia, pruritus, adenitis, arthralgia, and ocular inflammation.[16] Both the Mazzotti reaction and more moderate post-treatment reactions occurring after the first dose of ivermectin are associated with the release of intracellular *Wolbachia*.[15,17] In the natural course of infection, between 20,000 to 500,000 microfilariae die each day depending on the burden of infection, leading to significant chronic inflammation.[5]

There is a substantial time-lag between infection and symptom onset, which likely reflects the parasite's long pre-patent period of 7 months to 2 years.[18–20] Microfilariae are not typically detected in skin until 10-15 months after initial infection. The most common symptom of onchocerciasis is generalized pruritus, which ranges in severity from mild to unrelenting distressing itch.[21] Severe pruritus and dermatitis affect 6.5 million people with the disease, and can be disabling to the point of causing constant distress, interference with work and sleep, and even social stigmatization due to skin disfigurement.[5,18,22] In endemic communities, 40% of adults report troublesome itch.[21] A myriad of skin manifestations may occur in *Onchocerca* infection, and include papular rash, lichenification, hyper or hypopigmentation, and epidermal atrophy.[5,21] Onchocercal eye disease can occur in either the anterior or posterior chambers, and can present as uveitis, chorioretinitis, optic neuritis and punctate keratitis. Early corneal involvement is potentially reversible, however untreated eye disease may progress to fibrosing/sclerosing keratitis or optic nerve atrophy leading to permanent

visual impairment.[23] Other disease manifestations include lymphedema, lymphadenopathy, musculoskeletal symptoms, low body weight, and skin nodules.[24]

A complex interaction of host and parasite factors including intensity of infection, chronicity, *O. volvulus* strain, geographic area of acquisition, host genetics, and individual immune response appear to influence disease manifestations.[18,21,25–27] Although intensity of infection often is related to particular sets of clinical manifestations, in particular prevalence of anterior eye disease, it does not fully account for differences in symptoms.[18,25] Severe pruritus and extensive skin involvement can occur even with low parasite load, whereas individuals screened in endemic areas may be asymptomatic despite high microfilarial density.[18] Prolonged infection, although not the sole determinant of clinical presentation, is linked to both the presence of eye disease and specific skin manifestations including depigmentation and atrophy.[21] Early skin disease tends to be more inflammatory in nature, although inflammatory papules can be chronic, relapsing, or coexistent with other more chronic skin changes.[18]

Regional variations in clinical manifestations are also apparent within bioclimactic African zones, the Americas, and the Middle East. *O. volvulus* strains in West African savanna zones are associated with anterior segment ocular disease and a high prevalence of blindness, whereas West African forest strains have a propensity to cause skin disease and less frequently manifest with posterior segment eye involvement.[5] Although some regions harbour only one predominate strain, overlap occurs throughout savannah-forest transition zones. Patterns of disease in Central America are different than those observed in Africa, as endemic individuals in Guatemala and Mexico exhibit high rates of eye disease and numerous nodules, but less extensive

skin and lymph node involvement.[26] The relative distribution of onchocercal nodules appears related to biting patterns of different regional *Simulium* subspecies. Central American *S. ochracium* tends to bite on the upper half of the body, hence onchocercomata appear predominantly on the head and upper torso. African *S. damnosum* is a low-biter, with nodules often noted on the legs and inguinal areas, in addition to the upper body in those infected as children. Nodule location does not appear to be correlated with incidence of eye disease. It is unclear to what extent geographic differences in clinical presentation are mediated by *O. volvulus* strain, strain-specific *Wolbachia* burden, differences in local *Simulium* vectors, and host genetics.[27–29]

Finally, there is increasing evidence that the host's ability to modulate inflammatory responses to *O. volvulus* is a significant determinant of disease phenotype.[30] Human Leukocyte Antigen (HLA) genes, which are responsible for immunoregulation, differ in individuals with generalized disease, localized onchodermatitis, and putative immunity.[27,31] A dampening of immune response to *O. volvulus* specific antigens occurs in chronically-infected endemic individuals, and may be related to in-utero or neonatal exposure to *O. volvulus* antigen.[30,32,33]. Mother-to-child transmission of *O. volvulus* infection has occurred in endemic areas, and mothers with microfilaridermia secrete *O. volvulus* antigen in breast milk, providing potential means of early sensitization.[34,35]

Perhaps the most striking illustration of the complex interplay between geographic, genetic, and host-parasite immunoregulation is the unique form of hyperreactive onchodermatitis known as Sowda. Observed primarily in endemic populations in Yemen and Sub-Saharan Africa, Sowda presents as localized extensive

asymmetric skin disease with dark hyperpigmentation and pronounced regional lymphadenopathy.[36,37] Despite its dramatic skin manifestations, Sowda is characterized by a remarkable paucity of microfilariae, a robust immune response consisting of eosinophilia and high filaria-specific antibodies, and a more severe post-treatment reaction to ivermectin.[4,18,38] *O. volvulus* leads to a wide spectrum of clinical pathology through a variety of mechanisms, whose relative contributions are not yet fully established.

Comparative disease manifestations in endemic and non-endemic populations

With the rise in global migration and travel to remote destinations, filarial infections including onchocerciasis are increasingly diagnosed in non-endemic areas in immigrants, refugees and travelers.[39,40] Disease manifestations of many parasitic infections, including filarial infections, differ markedly between life-long residents of endemic areas and temporary residents who acquire the disease while abroad.[39,41,42] Travelers infected with the related filarial parasite *Loa loa* display dramatic acute symptoms consisting of Calabar swellings and urticarial rash. [41–43] This clinical phenotype is associated with immune hyperreactivity, as laboratory testing reveals marked eosinophilia, high parasite-specific immunoglobulins, and a shift towards Th2 cytokines in response to mitogen stimulation of peripheral blood mononuclear cells (PMBCs).[41,43] Despite a much higher burden of infection, endemic subjects with *Loa loa* or *W. bancrofti* display relative immune hyporesponsiveness, with comparatively few symptoms.[41,44]

Endemic individuals (END) and temporary residents (TR) with onchocerciasis have not been extensively compared. A 1994 study by McCarthy et al. noted significant

differences in clinical features, laboratory investigations, and immunologic markers between 20 TR and 21 END patients, although the assessment of the END was done in the field.[45] Onchocercal eye disease and subcutaneous nodules were notably absent in TR patients. Expatriates had a much lower burden of infection, as only 45% of infected TR had detectable microfiladermia compared with 100% of END ($P < 0.01$). The mean microfilarial density was only 1 (range 1-3) in TR, compared with 43 (range 1-115) in endemic subjects. Interestingly, the pattern of immunologic findings was the opposite of that observed in *Loa loa*: in *O. volvulus*, the END group had significantly higher eosinophil counts, and a more robust Th2 response with higher levels of IL-4 and IL-5 cytokines produced by mitogen-stimulated PMBCs. Unlike *Loa loa*, the differences in clinical manifestations between END and TR did not reflect a hyperresponsive state in the relatively immunologically-naïve TR group. The authors posited that chronicity or parasite load more likely influenced disease manifestations. However, this study had several notable limitations including small sample size, as well as potential confounding based on region of *O. volvulus* exposure and study setting. TR patients were all infected in West and Central Africa, and were evaluated at the National Institutes of Health, whereas END patients acquired infection in Guatemala and were studied under field conditions.

No other studies systematically compare END and TR with *O. volvulus* infection. Case series and clinical case reports provide insight into the clinical features of onchocerciasis in temporary residents, but provide no direct comparison to endemic individuals. Analyses performed by multi-national consortia of returned travelers

describe symptom timing and location of acquisition, but lack key details of the clinical assessment and relevant laboratory investigations. [39,40]

In most case series, *O. volvulus* infection in TRs occurs with prolonged travel of at least 3 months, although travelers occasionally acquire onchocerciasis during short trips to highly endemic areas. [23,40,45] In one multi-national study of returned travelers treated in subspecialty tropical disease clinics, three-quarters of *Onchocerca*-infected expatriates resided in the endemic area for less than 1 month. [39,46,47] Almost all travelers acquire *Onchocerca* in Africa, while travel-associated infection is extremely rare in Latin America.[40,48] Most studies and case reports describe delayed symptom onset ranging from months to years after leaving the endemic area, which is consistent with *O. volvulus*' prolonged pre-patent period.[18,23,45,49] However, the above multi-national analysis of returned travelers found that two-thirds of nonendemic visitors became symptomatic within 1 month of return from the filaria-endemic area. This finding suggests a shorter incubation period in TR than that previously described.[39] Significant delays in diagnosis are common, as medical practitioners in non-endemic areas are unfamiliar with onchocerciasis, and do not always relate symptoms to previous travel that occurred on average 1-3 years earlier.[18] Only the study of patients referred to specialized travel clinics achieved consistently rapid diagnosis within 1 month of return from the endemic area.[39]

Case series support McCarthy's observations that TR commonly present with pruritus or papular rash.[49,50] In 3 older cohorts of infected expatriates, pruritus was present in 68-89% of patients, and notably one-third had pruritus without evidence of rash.[49–51] Pruritus was localized to only the lower back or buttocks in two-thirds of a

cohort of travelers to West and Central Africa.[51] Rash was a presenting feature in half to two-thirds of patients, and was almost always pruritic and papular in morphology.[49–51] Few patients manifested lichenification or hyperpigmentation.[51] Small case series describe dramatic acute unilateral limb edema, in particular of the upper extremity, as a presenting feature unique to expatriates.[5,46,47,52]

The incidence of eye involvement in expatriates varies considerably in previous studies. Expert observations, case reports, and small case series support the finding by McCarthy et al. that onchocercal eye disease rarely occurs in expatriate populations. In a cohort of 22 infected expatriates returning from Cameroon, only 1 had evidence of punctate keratitis.[49] However, two much larger historical expatriate cohorts challenge this assertion. In a study of 95 Dutch expatriates with onchocerciasis conducted from 1959-1971, Smit et al. found evidence of punctate keratitis in 13 of 59 travelers (22%) who received an ocular exam.[50] Similarly, Woodruff et al. noted ocular findings in 34% of a cohort of 76 patients, of which 72 were European expatriates. In both studies, the lack of ocular symptoms did not preclude eye involvement. Woodruff et al. found ocular microfilariae in 8 entirely asymptomatic patients, and only 23% of expatriates with punctate keratitis complained of eye symptoms.[51]

Differences in duration of both exposure and infection might explain the drastically different burden of eye disease observed in more recent and comparatively historical TR cohorts. The mean duration of residence in an *O. volvulus* endemic area was 11.6 years in the study by Woodruff et al., in which TR commonly had eye disease. By contrast, McCarthy observed no eye disease in TR, whose median exposure duration was only 2 years (range 20 months to 6 years). Pryce et al. observed only 1 case of

punctate keratitis in a cohort exposed for a mean of 3 months (range 2 days to 15 months). Woodruff et al. asserted that exposure duration did not explain the high prevalence of eye disease in their patients, based on a shorter mean exposure of 8.2 years in the group with ocular disease, in comparison to the rest of the cohort. Regardless, it is remarkable that the mean exposure in Woodruff et al. was longer than the maximum duration of residence in the two cohorts with low prevalence of eye disease. Furthermore, a subset of patients in the Woodruff et al. study acquired onchocerciasis in the pre-treatment era, and had prolonged untreated infection for 15-20 years. This more closely parallels the chronic *O. volvulus* infection seen in endemic areas, and could therefore account for ocular involvement on par with local populations.

High burden of infection was associated with onchocercal eye disease in the McCarthy et al. cohort, but microfilarial density did not predict eye involvement in older case series. Woodruff et al. noted onchocercal eye disease in one third of lightly infected expatriates who had no evidence of dermal microfilariae. Regional differences in *O. volvulus* strain could have contributed to the varying prevalence of eye disease observed between studies. TR patients where almost exclusively infected in West or Central Africa in all four studies, but information about specific location of acquisition was not sufficiently precise to determine whether infections occurred in forest, savannah or forest-savannah mosaic zones. In the two studies showing a low prevalence of eye disease, almost all TR were infected in either Sierra Leone or Cameroon.[45,49] Older studies that observed substantial eye disease included expatriates infected primarily in Cameroon and Nigeria. Prevalence of eye disease differs dramatically on a regional basis, such that it is not possible to draw conclusions about *O. volvulus* strain or

comparative infection intensity at the country-level. For instance, even within Sierra Leone, community prevalence of *O. volvulus* eye disease ranges from 4% in typical forest zones to 75% in some savannah villages. [53,54]

Lastly, the relative prevalence of asymptomatic *O. volvulus* infection in END and TR is not established. On one hand, it is plausible that immunologically naïve expatriates without prior exposure to parasite antigen could experience more symptoms despite a much lower parasite load. In a GeoSentinel analysis of returned travelers with filarial infection, patients born in filaria-endemic areas were 2.5 times more likely to be asymptomatic, as compared with non-endemic visitors (95% CI 1.07-5.81, $P = 0.03$).[39] However, this study included patients with all filarial infections, of which only 37% had onchocerciasis. It is equally plausible that an extremely low parasite burden might cause no or minimal symptomatology. Of 22 travelers diagnosed with onchocerciasis after an expedition to Cameroon, 9% were entirely asymptomatic. The prevalence of asymptomatic *O. volvulus* in TR may have been even higher, as only 60% of cohort members received diagnostic testing.[49] Similarly, 16 of 76 (21%) expatriates with microfilaridermia were asymptomatic in the study by Woodruff. Interestingly, duration of exposure was longer in asymptomatic patients.

Comparing immunologically naïve temporary residents and immune tolerant endemic populations provides a means of understanding how host-parasite interactions contribute to clinical pathology. Few studies address this relationship in *O. volvulus* infection. Although case series of *O. volvulus* infected travelers provide useful descriptive information, they offer no direct comparison with disease in endemic populations. We aimed to systematically compare symptom timing, clinical presentation,

physical examination findings, and laboratory findings associated with *O. volvulus* infection in endemic individuals and temporary residents evaluated in a non-endemic tertiary care setting. By characterizing disease manifestations in these two populations, we enable health care providers to better recognize diverse presentations of onchocerciasis in non-endemic areas. Furthermore, we hoped to determine whether the paradigm of parasite-induced immune hyperresponsiveness in TR observed in *Loa loa* also holds true in onchocerciasis.

METHODS

Study subjects

We included all patients with active *Onchocerca volvulus* infection evaluated by the Clinical Parasitology Section of the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases (NIAID) between 1976 and 2016. Patients with active onchocerciasis had prior exposure to an *O. volvulus*-endemic region, and met at least one of the following criteria: microfilaria visualized on "skin snips" or slit lamp examination; *O. volvulus* DNA detected by polymerase-chain reaction (PCR) testing of "skin snips"; positive antifilarial antibody testing, in addition to either a positive Mazzotti provocation test with diethylcarbamazine or a characteristic post-treatment reaction; or, positive antifilarial antibody testing in association with characteristic clinical symptoms of *O. volvulus* infection.[40,55] All patients underwent "skin snips" and antifilarial antibody testing. Only a subset of patients received Mazzotti provocation testing, which was historically used to aid in diagnosing patients with suspected infection, a normal slit lamp examination, and without evidence of dermal microfilariae. Mazzotti provocation

testing was employed only in the earlier part of the cohort, and abandoned as a means of diagnosis in all patients evaluated at the NIH after the year 2000.

Research Ethics

The study was conducted under protocols approved by the NIAID Institutional Review Board, including the registered protocols NCT00001230 and NCT00001645. All patients gave informed consent to participate. Characteristics of a subset of temporary residents in this study were previously described.[45,55]

Data collection and verification

The lead author (AJS) retrospectively abstracted patient data from all available electronic medical records and paper charts, using a standardized electronic data abstraction form developed *a priori* (Appendix 1). We recorded baseline demographic information, clinical signs and symptoms both at baseline and throughout the treatment course, routine and parasite-specific laboratory investigations, and antifilarial drug treatment. We performed range edits and value checks to reduce the potential for data entry errors, and verified the accuracy of aberrant values.

Patient Evaluation

At study entry, all patients received a detailed medical history including travel history, and a complete physical examination. Clinical assessments were performed by NIAID Laboratory of Parasitic Diseases (LPD) physicians with experience in the diagnosis and management of parasitic infections. Patient evaluations took place both in

the outpatient department and in the NIH inpatient unit. Baseline evaluation included a dilated slit lamp examination performed by an ophthalmologist to exclude onchocercal eye disease.

Laboratory investigations and parasitologic testing

All patients received baseline laboratory investigations including a complete blood count with differential (WBC; normal range, 4.23 – 9.07 cells/mL), metabolic panel, urinalysis, and quantification of immunoglobulin G (IgG; normal range, 700–1500 mg/dL), immunoglobulin M (IgM; 60–300 mg/dL), immunoglobulin A (IgA; 60–400 mg/dL), and immunoglobulin E (IgE; 3–423 IU/mL).[41]

Skin snips were performed on all patients to visualize dermal *O. volvulus* microfilariae by microscopy and/or to detect *O. volvulus* DNA through PCR. Skin snips were performed using standard technique, using a Holth-type corneoscleral punch (Storz Instruments, St. Louis, Missouri) with samples taken from bilateral shoulders, hips and thighs.[45] Patients received stool ova and parasite testing to identify coinfection with intestinal helminths, and urine ova and parasite testing for schistosomiasis when appropriate. Patients with potential exposure to *Loa loa* underwent Nuclepore™ filtration (Pleasanton, California) of 1 mL of whole blood collected between 10 and 2 pm, and/or *Loa loa* PCR testing of whole blood.[41] Patients with suspected *Wuchereria bancrofti* infection had Nuclepore™ filtration of whole blood collected between 10 pm and 12 am, and/or circulating antigen testing using the TropBio enzyme-linked immunosorbent assay (JCU Tropical Biotechnology Private Limited, Queensland, Australia).[41,56] Additional laboratory and radiologic investigations, including testing

for non-parasitic coinfections such as HIV and hepatitis, were performed at the discretion of the treating physician.

Parasite-specific immunoassays and recombinant antigen testing

Testing for antibody response to a crude extract of filarial antigens and recombinant filarial-specific proteins was performed on patient sera stored at -80 degrees Celsius, and from the earliest study time-point available. Antibody responses to *Brugia malayi* adult antigen (BMA) is measurable in all types of filarial infection because of its extensive antigenic cross reactivity with each of the filarial species; it is highly sensitive but not specific for *O. volvulus* infection.[45] IgG and IgG4 antibodies to crude extracts of *Brugia malayi* adult antigen (BMA) were measured by ELISA using a standard technique as described elsewhere.[57,58] A BMA-IgG >=14 μg/mL or BMA-IgG4 > 0 ng/mL was considered a positive test. Normal values were defined based on the upper 99% confidence limits for 62 unexposed North Americans. [55,57]

Antibody responses to the recombinant filarial antigens *Wb*-123, *Ll*-SXP-1, and *Ov*-16 are highly specific, but somewhat insensitive markers of infection with *Wuchereria bancrofti,* [59] *Loa loa*, [60] and *Onchocerca volvulus* respectively.[61] We performed additional testing for serum antibodies to *Wb*-123, *Ll*-SXP-1, and *Ov*-16 using the Luminex™ platform (Bio-Plex MAGPIX Multiplex Reader; Bio-Rad). For measurement of parasite-specific IgG4, we created 1:50 dilutions using 5 μL of thawed patient serum and 245 μL ELISA diluent (PBS Tween 1% BSA). To measure IgG, we used a 1:1000 dilution using 10 μL of the 1:50 dilution, combined with an additional 190 μL of ELISA diluent. We aliquoted 100 μl each of IgG and IgG4 into separate 96-well

microplates (Greiner Bio-One). We used a calibrated standard curve with known concentrations of antibody to recombinant parasite antigen.

Parasite antigens *Wb*-123, *Ll*-SXP-1, and *Ov*-16 were purified exactly as previously described.[45,59,60] Recombinant antigens were then chemically bound to magnetic Luminex™ beads using 10 mcg of each antigen and 25 million beads. Bead sets were vortexed for 1 minute, then placed in an ultrasound bath for 1 minute. We then diluted 4 μL of each bead region (25 million beads/mL) in 5 mL of ELISA diluent, and added 50 μL of bead dilution to each well (1000 beads/well) containing patient sera. Plates were then sealed, shaken at 500 rpm for 1 hour at room temperature, and washed 3 times with 100 μL of PBS Tween, with intervening 1 minute soaks (Biotek 405 microplate washer).

We then added 100 μL of 1:000 dilution of biotin-conjugated IgG antibody (Jackson ImmunoResearch) to each IgG well, and biotin mouse anti-human IgG4 (Southern Biotech) to each IgG4 well. Plates were again shaken at 500 rpm for 1 hour, and washed as above. We then added 50 μL of 1:500 PE streptavidin (Jackson ImmunoResearch) per well. Plates were shaken at room temperature for 15 minutes, and underwent a final wash. An additional 80 μL of ELISA diluent was placed in each well, plates were shaken for 10 minutes at 500 rpm at room temperature, and then read on the Luminex system. Signal to noise ratios were interpreted based on a positive cut-off of >=20 for IgG and >=5 for IgG4.

Definitions

Patients born in an *Onchocera*-endemic country were classified as endemic individuals (END), whereas those born in a non-endemic country were designated as temporary residents (TR). We calculated duration of exposure for all patients with known dates of entry and exit into *O. volvulus* endemic countries. For patients with multiple discrete visits to an endemic area, the duration of exposure represented the cumulative duration. For the END group, the patient's birth date was the date of entry. Time from exit to symptom onset was defined as the time between the last possible exposure to *O. volvulus* and the date of symptom onset. This was calculated only for the subset of patients who became symptomatic after leaving the endemic area. Time to diagnosis was defined as the time between symptom onset and diagnosis of *O. volvulus* infection, at the NIH or by another physician prior to the NIH baseline visit.

Asymptomatic patients reported no characteristic symptoms of onchocerciasis, including pruritus, rash, visual disturbance, limb swelling, arthralgias, and nodules. Eosinophilia was defined as an absolute eosinophil count $\geq$ 500 cells/mL. Leukocytosis was defined as a baseline white blood cell count > 9.07, which is the upper limit of the normal range for the Clinical Pathology Laboratory at the NIH.

Statistical Analysis

Categorical variables were compared using Fisher's exact test. Unless otherwise specified, continuous variables were analyzed using non-parametric testing with the Mann-Whitney U test. When continuous data followed a normal distribution, the student's t-test was used. We used geometric means with 95% confidence intervals as measures of central tendency unless otherwise indicated.

Univariate and multivariate linear regression analyses were performed to evaluate the association between two key outcomes (absolute eosinophil count (AEC) and BMA-IgG4 level) and covariates including sex, duration of exposure to the endemic area, and coinfection with other helminth/filarial parasites. The distribution of AEC and BMA-IgG4 were positively skewed, therefore a logarithmic transformation was performed in the analysis. Covariates were chosen based on the *a priori* hypothesis of potential confounding of the relationship between endemicity and the outcome of interest. Testing for normality was performed using Quantile-Quantile plots, Kernel Density estimates, and the Shapiro-Wilk test. Model residuals followed a normal distribution according to the Shapiro-Wilk test. Colinearity was tested by calculating variance inflation factors, which were all less than 10. The final multivariable regression model was analyzed based on robust resistant regression with bootstrapping performed with 1000 repetitions, to account for both non-normally distributed data and outliers. Univariate logistic regression was performed to examine patient risk factors for onchocercal eye disease. Data were analyzed using Stata statistical software version 13 (StataCorp LP, College Station, Tx).

RESULTS

Patient characteristics

We identified 76 patients with active *O. volvulus* infection, including 40 TR and 36 END patients. Fifteen END and 21 TR met inclusion criteria based on the presence of microfilariae or *O. volvulus* DNA in skin snips, 5 END and 24 TR had a positive

Mazzotti test/post-treatment reaction along with positive antifilarial serology, and 36 END and 36 TR had symptoms of *O. volvulus* infection in addition to positive antifilarial serology.

TR patients were more likely to be male (27/40 [68%]) compared with those in the END group (15/36 [42%], $P = .037$) (Table 1). Median age was 33.3 years in the entire cohort (range 3.2-67.8 years), with no difference between groups ($P = .12$). Almost all END patients were African or African American (35/36 [97.2%]), while most TR were Caucasian (37/40 [92.5%], $P < .001$). The majority of patients in both groups acquired infection in West or Central Africa (70/76 [92%]). END patients were mainly infected in Cameroon (27/36 [75%]), while TR patients acquired infection throughout West and Central Africa, most commonly in Sierra Leone (14/40 [35%]). The TR group included 25 Peace Corps volunteers, 5 missionaries, 4 research scientists, 2 healthcare workers, 2 children, and 2 occupational travelers. *O. volvulus* infection occurred after a single period of exposure in 79% (16/76) of patients, with no major difference between TR and END groups (29/40 [72.5%] vs 31/36 [86.1%], $P = .17$). The median duration of exposure to an *O. volvulus*-endemic area was significantly shorter in TR patients (28 months [range 2 – 207], compared with END patients (373 months [range 12 - 823], $P < .001$).

Coinfections

Concurrent parasitic infections were common in both groups. Filarial coinfection, all with *Loa loa*, was present in 11.1 % of END and 5% of TR ($P = 0.41$). No patient was infected with *Wuchereria bancrofti* or *Mansonella spp*. Seven END (19.4%) and 3

TR (7.5%) were infected with at least 1 soil-transmitted helminth (P = .18). The most common helminth coinfections were *Strongyloides stercoralis* (5 patients), hookworm (3 patients), and *Trichuris trichiura* (1 patient). One patient was coinfected with *S. stercoralis* and hookworm. Two END (5.6%) and 1 TR (2.5%) had schistosomiasis (P = .6). Other coinfections diagnosed at baseline included *Giardia lamblia* (2 TR, 2 END), chronic active hepatitis B (1 TR, 3 END), Hepatitis C (1 END), latent tuberculosis (2 END), active pulmonary tuberculosis (1 END), and hyperreactive malarial splenomegaly (1 END). No patient was diagnosed with HIV.

Symptom timing and characteristics

Most TR patients developed symptoms of *O. volvulus* infection after repatriation (29/35 [82.9%]) whereas the majority of END patients (18/29 [62.1%]) first noted symptoms prior to leaving the endemic area (Table 1). In those who became symptomatic in the United States, the time interval between exiting the endemic area and symptom onset was prolonged in both groups: TR manifested initial symptoms a mean of 15 months (SD 11.2) after leaving the endemic area, while END developed symptoms a mean of 33.5 months after immigration (SD 31.0, P = .096). Although substantial delays in *O. volvulus* diagnosis occurred in both groups, END patients experienced more prolonged symptoms prior to diagnostic confirmation. The median time from symptom onset to *O. volvulus* diagnosis was 6.3 months (IQR 2.8 – 15.2) in TR patients, and 26.9 months (IQR 12.0 – 53.8) in the END group (P < .001).

Symptoms prior to any filarial treatment were documented for all TR and 31 END (86.1%), and are described in Table 2. Five TR patients (12.5%) were entirely

asymptomatic, whereas all END patients manifested at least one characteristic symptom of *O. volvulus* infection ($P = .064$). The majority of both TR and END had pruritus (TR 32/40 [80%] vs END 28/31 [90.3%], $P = .33$). TR were more likely to describe rash (26/40 [65%]) compared with END (7/31 [22.6%], $P = .001$). No TR complained of visual disturbance, which occurred in 12.9% of END patients ($P = .032$). Three TR (7.5%) and 3 END (9.7%) had arthralgias ($P = 1.0$). Approximately 23% of patients in both groups described limb swelling ($P = 1.0$). Of those who reported limb swelling, most (81%) had no evidence of *Loa loa* coinfection.

Physical examination

All patients received a physical examination at the baseline study visit (Table 3). Although similar numbers of TR and END had an abnormal dermatologic examination (TR 22/40 [55.0%] vs END 20/36 [55.6%], $P = 1.0$), skin manifestations were different in the two groups. Papular onchodermatitis was present in 47.5% (19/40) of TR, but only in 2.7% (1/36) of END ($P < .001$). Skin pigmentation changes occurred more frequently in END patients (15/36 [41.7%]), compared with the TR group (6/40 [15%], $P = .01$). Lichenification was observed more often in END patients (5/36 [13.9%]) than in TR (1/40 [2.5%]), but these differences were not statistically significant ($P = .096$). One END patient in the series had evidence of hyperreactive onchodermatitis (Sowda) manifesting as papules, lichenification, and hypopigmentation.

Onchocercal eye disease occurred almost exclusively in the END group. Among END patients who received slit lamp examination, 7 (22.6%) had documented abnormalities consistent with sequelae of *O. volvulus* infection. Only 1 TR (3.3%) had

evidence of onchocercal eye disease. The difference approached statistical significance (P = .053). Slit lamp examination was not documented in 5 END and 10 TR patients. Specific ocular findings in the END group included corneal scarring in 4 patients, punctate keratitis in 2 patients, and sub-retinal scarring in 1 patient. One TR had evidence of corneal scarring and retinal folds. No patient in either group had posterior eye lesions. Univariate analysis revealed no difference in the odds of onchocercal eye disease based on sex (OR 0.7 [95% CI .15-3.1], P = .6) or region of exposure (West Africa vs. Central Africa, OR 3.9 [95% CI .4-36], P = .2). The odds of onchocercal eye disease increased slightly with each additional year of residence in the endemic area (OR = 1.05 [95% CI 1.0 – 1.11], P = .04). The TR patient with *O. volvulus* eye disease was the only person infected in East Africa after an unknown duration of residence.

Unilateral limb edema was present in 1 END (2.8%) and 5 TR (12.5%, P = .2). Overall, 5 patients had unilateral upper extremity edema, and 1 had unilateral lower extremity edema. Only 1 TR with upper extremity edema had concurrent filarial infection with *Loa loa*. Similar proportions of END and TR had lymphadenopathy (END 4/36 [11.1%] vs TR 7/40 [17.5%], P = .52) and subcutaneous nodules (END 6/36 [16.7%] vs TR 4/40 [10%], P = .5). All patients with documented subcutaneous nodules had only 1 or 2 palpable nodules. Of the 4 TR patients with nodules, 3 had nodules on the upper body (head, epitrochlear, wrist), and 1 had trochanteric and iliac crest involvement. In the END group, nodules were located on the upper chest wall or back in 3 patients, upper limb in 1, lower limb in 1, and iliac crest/buttocks in 1 patient.

Baseline physical examination took place prior to treatment at the NIH, however some patients had previously received antifilarial medications administered elsewhere.

There was no difference in the proportion of patients who had received antifilarial treatment within 5 years prior to the baseline physical examination (TR 10/40 [25%] vs END 13/36 [36.1%], $P = .33$).

Laboratory investigations

Table 4 describes baseline laboratory investigations for all patients who had not received antifilarial treatment within 5 years of study entry. Absolute eosinophil counts (AEC) were similar for both TR (GM 797.5 cells/mL [95% CI 502.1-1266.7]) and END (GM 763.7 cells/mL [95% CI 432.9 - 1347.1], $P = .97$) (Figure 1). Multivariable regression analysis also revealed no difference in AEC comparing END and TR, adjusting for sex, duration of residence in the onchocerca-endemic area, and presence or absence of helminthic coinfection ($P = .2$). None of these variables were independently associated with AEC in our cohort. Only two-thirds of patients in each group had eosinophilia. There was no difference in eosinophil percentage (END 13.6 [95% CI 8.6 - 21.7] vs TR 11.8 [95% CI 8.1-17.3], $P = .47$). The TR group had a higher geometric mean white blood cell count (7.3 x 10^3/mL [95% CI 6.4 - 8.5]) than the END group (5.7 x 10^3/mL [4.9 - 6.6], $P = .003$), although there was no difference in the proportion of patients with leukocytosis (2/21 [9.5] in END and 4/28 [14.3%] in TR, $P = .69$).

END patients had higher baseline polyclonal IgG (GM 1968 mg/dL [95% CI 1590 - 2436]) compared with the TR group (GM 1233 [95% CI 1147 - 1324], $P = .001$). Polyclonal IgG was elevated in 80% of END, but in only 4.5% of TR ($P < .001$). Polyclonal IgE was also significantly higher in END compared with TR (GM 1309 IU/mL [95% CI 652 - 2628] vs 251 [95% CI 139 – 451], $P < .001$); however, there was

no difference in the proportion with a polyclonal IgE above the normal range (END 15/20 [75%] vs TR 11/23 [47.8%], *P* = .12).

Parasite-specific serologic testing

All but 3 patients (2 TR, 1 END) had an elevated BMA-IgG or BMA-IgG4 (Table 5). Geometric mean BMA-IgG concentrations were similar in END (158 μg/mL [95% CI 100 - 250]) and TR groups (175 μg/mL [95% CI 11 2 - 275], *P* = .68); however, the IgG-specific percentage of the total IgG (BMA-IgG/total IgG) was significantly higher in TR than END (1.7% [95% CI 1.0- 2.8] vs 0.8% [95% CI .4-1.5], respectively, *P* = .04) (Figure 2). Patients in the END group were more likely to have positive testing for BMA-IgG4 (89% vs 62.5%, *P* = .015), and among those who tested positive, END patients had significantly higher BMA-IgG4 levels (7916 ng/mL [95% CI 5167 - 12128]) compared with TR (2241 ng/mL [95% CI 1171 - 4290], *P* = .005). There was no significant difference in BMA-IgG4 comparing END and TR after adjusting for sex, duration of exposure, and presence of filarial coinfection (*P* = .7). Although duration of exposure was positively associated with BMA-IgG4 level on univariate analysis (*P* = .03), this effect was not observed after multivariate regression (*P* = .9). A higher proportion of END patients were positive for *Ov*-16 IgG4 (END 18/36 [50%] vs TR 10/40 [25%], *P* = .03), but there was no difference between groups in geometric mean *Ov*-16 IgG4 (END 676 ng/mL [95% CI 343 - 1333] vs TR 618 ng/mL [95% CI 218 - 1750], *P* = .84).

DISCUSSION

We evaluated 40 temporary residents and 36 endemic individuals with onchocerciasis who underwent rigorous diagnostic testing in a non-endemic tertiary care setting. Although there was substantial overlap in the presentation of onchocerciasis in TR and END, our study revealed several important differences between these two groups. Both END and TR were equally likely to present with skin manifestations, but the nature of skin involvement differed: the TR group had more papular dermatitis, whereas END had pigmentation changes which are typically associated with more chronic inflammation. Eye disease occurred in only 1 TR, whereas 23% of END patients had ocular involvement based on slit lamp examination. There was a trend towards a higher prevalence of asymptomatic infection in TR patients, of which 12.5% had no characteristic symptoms of *O. volvulus*.

Unlike *Loa loa*, differences between END and TR infected with *O. volvulus* did not appear to be related to an eosinophil-mediated immune hyperresponsive state in TR. Our study found no difference whatsoever between groups in either absolute eosinophil count or eosinophil percentage. We observed the same result after controlling for sex, helminth coinfection, and duration of exposure to the endemic area, which were therefore not confounding variables. Our results differed from those of McCarthy et al., who observed significantly higher absolute eosinophil count in END (GM 2634 cells/mL [95% CI 268 - 7342]) compared with TR (GM 1056 cells/mL [95% CI 250 -3245], $P < 0.05$). Notably, the geometric mean eosinophil count in END patients in the McCarthy study was well above the upper limit of the 95% confidence interval for our END group. Discordant results might be attributed to the very different composition of the END groups. We evaluated infected endemic individuals presenting in a non-endemic setting,

on average years after leaving the endemic area, whereas McCarthy et al. looked at END patients within an endemic setting. END patients in the earlier study had ongoing *O. volvulus* exposure, and presumably also a much higher prevalence of geohelminth infection, both of which might raise eosinophil count.[45]

Differences in END and TR may instead be linked to infection chronicity. END subjects had a significantly longer duration of exposure and time to diagnosis, which implies more prolonged untreated infection. Onchocercal eye disease was associated with longer exposure duration in the END group. END patients also had higher levels of BMA-IgG4, and more frequent *Ov*-16 IgG4 positivity, both of which likely reflect more chronic disease. Several other measured and unmeasured variables may also have contributed to differences observed between groups. END and TR differed based on both sex and race. Microfilarial densities were not well documented in many patients with microfilaridermia, so we could not evaluate the impact of parasite burden. We also could not determine whether there were clinically important between-group differences in *O. volvulus* strain. Almost all patients in both groups acquired onchocerciasis in Africa, but more TR patients were infected in West Africa (Sierra Leone), and most END acquired *O. volvulus* in Central Africa (Cameroon). *O. volvulus* strains prevalent in West African forest zones are known to cause less eye disease. However, there is substantial intra-country *O. volvulus* strain variability within forest, savannah and transition zones, and this degree of regional geographic information was not available in our cohort.[29]

Although we could not establish the causal mechanism responsible for differences between END and TR, our findings have important implications for clinicians in non-endemic countries. First and foremost, physicians must consider *O. volvulus* based on

relevant epidemiologic exposure alone, as one eighth of TR were entirely asymptomatic. These patients were typically referred for evaluation after an incidental finding of eosinophilia, or were screened based on similar exposure to a known *O. volvulus* case. The substantial number of asymptomatic expatriates also supports an approach of active case-finding within cohorts of travelers with similar epidemiologic exposures.[49] Interestingly, there was a trend towards a higher prevalence of asymptomatic infection in TR patients compared with the END group. Screening programs in endemic areas commonly identify asymptomatic infection in endemic areas, but all END patients in our cohort had at least one symptom of onchocerciasis.[5] This unexpected finding may be related to health-care seeking behaviour of foreign-born populations in a non-endemic setting. Time to diagnosis was substantially longer in the END group compared with TR, which may reflect lack of access to healthcare or prioritization of other needs in the context of recent immigration.

Our study also has implications for the timing of recommended physician evaluation post-travel or immigration. *O. volvulus* symptoms manifested months to years after return from the endemic area. Most TR presented between 6 to 12 months after return, and therefore symptoms would be missed at a one-time screening medical examination for tropical infections that typically occurs between 1 to 2 months after repatriation, if at all. For TR, a second travel-focused medical examination occurring 1 year after return might increase detection of *O. volvulus*. The time-lag was even longer for END patients, who presented for medical care a median of ~3 years after immigration to the United States. Education of primary care providers serving populations from endemic areas might be a more effective strategy in this population, as the longer time to

symptom onset requires serial evaluation in the first 5 years after immigration. The above interventions could reduce the unacceptably long median time to diagnosis of 6 months in TR and 2 years in END.

The lack of eye disease in TR in our study corroborates findings of McCarthy et al., recent case series of returned travelers, and expert opinion.[5,45] The low prevalence of eye disease in our TR group compared with historical cohorts may reflect differences in infection chronicity or intensity. TR in our series spent a median duration of 2 years in *O. volvulus* endemic areas, and had untreated infection for approximately 6-12 months before a diagnosis was established. In historical cohorts, TR populations with a high prevalence of onchocercal eye disease spent a median of 8-11 years in *O. volvulus* endemic areas and had chronic untreated infection more similar to our END population.[50,51] Unless exposure is very prolonged, TR patients appear unlikely to develop eye disease. Unfortunately, duration of exposure was unknown for the sole TR in our series with *O. volvulus* eye involvement. A 23% prevalence of onchocercal eye disease in our END group strongly supports routine slit lamp examination in migrants from highly endemic areas, in particular those who report visual symptoms.

Although eye disease is rare in TR, we found that unilateral limb swelling is a fairly common presenting symptom and should prompt consideration of *O. volvulus*. [46,47] One fifth of TR reported limb swelling, and one eighth of TR had objective unilateral limb edema on baseline physical examination. Limb swelling in 1 TR could have been related to *Loa loa* coinfection, but the remaining 4 TR patients were not infected with *Loa loa* or *Wuchereria bancrofti*. In contrast to prior studies, we observed skin nodules on physical examination in equal proportions of END and TR. Prior case

series specifically remark on the absence of nodules in expatriates. [45] Although physical examination in our series was performed by experienced physicians with training in parasitic diseases, nodules palpated on exam were not confirmed to be onchocercomata based on biopsy, excision, or imaging. We could therefore not exclude other causes such as fibromas, lymph nodes, or ganglion cysts.

Finally, our study has implications for laboratory testing for *O. volvulus* in returned travelers and migrants from endemic areas. We found that a normal eosinophil count cannot be used to rule out *O. volvulus* infection in either END or TR. One-third of *O. volvulus*-infected patients had a normal eosinophil count. In common clinical practice, BMA-IgG4 is the only test performed to screen for suspected filarial infection. In our study, relying on BMA-IgG4 alone to exclude *O. volvulus* infection would have missed 10% of infections in END and 40% of infections in TR. BMA-IgG4 is linked to more chronic infection, and may not be elevated in TR presenting in the acute setting. *Ov*-16 IgG4 was not a sensitive test and was elevated in only half of END and one quarter of TR, which is a noteworthy limitation when this test is employed as a screening tool.

Limitations

Although this is the largest study to date directly comparing *O. volvulus* infection in endemic individuals and temporary residents, onchocerciasis is a rare diagnosis in a non-endemic setting and the number of study subjects was small. Some patients in both groups had received prior treatment with antifilarial drugs, which can affect both eosinophil count and parasite-specific antibody response. In comparing laboratory

investigations, we therefore looked exclusively at the patient subset who had not been treated within 5 years, which further reduced our sample size.

There is also the potential for selection bias, as patients referred for evaluation and treatment at the National Institutes of Health may differ from the broader population with *O. volvulus* in the United States. In the earlier part of the cohort, difficult access to antifilarial drugs to treat onchocerciasis mitigated the potential for selection bias, as most patients were treated in highly specialized tertiary care centers similar to the NIH. The McCarthy et al. study published in 1994 examined a subset of TR included in our cohort, and determined this to be a representative sample of patients with *O. volvulus* in the United States.[45] The FDA approval of ivermectin in 1998 allowed for widespread use without requiring an Investigational New Drug application through the Center for Disease Control. Patients treated at the NIH in the more recent part of the cohort could therefore potentially represent either more refractory or severe cases than those treated in the broader community setting.

Although patient assessment occurred prospectively according to established NIH research protocols for the evaluation and treatment of parasitic infections, we abstracted patient information retrospectively using existing medical records in which documentation was at times incomplete. We were therefore unable to systematically collect data pertaining to microfilarial density, which was not always recorded in the patient chart at the time of skin snips. All patients received a full physical examination performed by an experienced Laboratory of Parasitic Diseases physician, however there was still the potential for observer variability particularly in the description of *O. volvulus* skin manifestations. Although an ophthalmologist performed a slit lamp examination for

all patients as part of the routine study protocol, documentation of the assessment was missing for a total of 10 TR and 5 END, whose examinations were mostly likely normal. There remained a trend towards fewer ocular manifestations in TR, but with complete data the difference between groups would have most likely reached the $P < .05$ threshold for statistical significance.

Conclusions

The global burden of onchocerciasis continues to decrease as a result of vector control programs and mass drug administration campaigns, which are now entering the elimination phase in many countries.[7] Onchocerciasis however remains a major public health problem in areas of West Africa that are co-endemic for *Loa loa*. Ivermectin mass drug administration cannot be broadly implemented in co-endemic areas, due to the risk of precipitating encephalopathy in individuals with high levels of *Loa loa* microfilaremia. Although point-of-care diagnostics for *Loa loa* may ultimately resolve this problem, both endemic populations and travelers are still at risk of infection.[62,63]

Our study describes differing clinical presentations of onchocerciasis in migrants and travelers. Unlike *Loa loa*, differences in END and TR are not eosinophil mediated, and could instead be related to infection chronicity. Comparison of Th1 and Th2 cytokine production in response to parasite antigen could shed light on the mechanism underlying the clinical differences we observed. The duration of treatment required to achieve clinical cure in a non-endemic setting is also unknown.[55] Further evaluation of clinical, eosinophil and parasite-specific antibody response to treatment in our patient cohort is ongoing.

Table 1. Demographic characteristics of Endemic Individuals and Temporary Residents with Onchocerciasis

Characteristic	Endemic Individuals n = 36	Temporary Residents n = 40	*P* Value
Female, No. (%)	21 (58.3)	13 (32.5)	.037
Age, years, median (IQR)	36.9 (26.7-45.2)	30.3 (27.5-38.6)	.12
Race, No. (%)			<.001
Caucasian	1 (2.8)	37 (92.5)	
Africa or African American	35 (97.2)	3 (7.5)	
Region of acquisition, No. (%)[a]			.62
West/Central Africa	35 (97.2)	35 (87.5)	
East Africa	0	1 (2.5)	
Africa, multiple regions	0	2 (5)	
Central/South America	1 (2.8)	2 (5)	
Loa loa coinfection, No. (%)	4 (11.1)	2 (5.0)	.41
Intestinal helminth coinfection, No.(%)[b]	7 (19.4)	3 (7.5)	.18
Helminth coinfection, No. (%)[c]	12 (33.3)	6 (15)	.1
Duration of residence in endemic area, months, median (IQR)[d]	373 (295-520)	28 (24-48)	<.001
Symptom onset in endemic area, No. (%)[e]	18 (62.1)	6 (17.1)	<.001
Time to symptom onset after leaving endemic area, months, mean (SD)[f]	33.5 (31.0)	15 (11.2)	.096
Time from symptom onset to diagnosis, months, median (IQR)[g]	26.9 (12.0-53.8)	6.3 (2.8-15.2)	<.001

Abbreviations: IQR, interquartile range; SD, standard deviation;

[a] West/Central Africa included Benin, Gambia, Guinea, Ghana, Ivory Coast, Liberia, Nigeria, Senegal, Sierra Leone, Togo, Angola, Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Equatorial Guinea, and Gabon; East Africa included Burundi; Central/South America included Guatemala and Venezuela.
[b] The most common intestinal helminths were *Strongyloides stercoralis* (5 patients), Hookworm (3 patients), and *Trichuris trichiura* (1 patient). One patient was coinfected with *S. stercoralis* and Hookworm.
[c] Included infection with other filarial parasites, soil-transmitted helminths, and schistosomiasis
[d] Unable to determine for 6 END and 7 TR patients.
[e] Excluded 4 asymptomatic TR. Symptom onset location was unknown for 1 TR and 7 END.
[f] Calculated using the student's t-test, based on 10 END and 22 TR patients.
[g] Included 23 END and 24 TR patients with documented symptom onset and diagnosis dates.

Table 2. Baseline Symptoms Prior to Antifilarial Treatment

Symptom [a]	Endemic Individuals No. (%)	Temporary Residents No. (%)	*P* value
Asymptomatic	0 (0)	5 (12.5)	.064
Pruritus	28 (90.3)	32 (80)	.33
Rash	7 (22.6)	26 (65)	.001
Visual disturbance	4 (12.9)	0 (0)	.032
Limb swelling	7 (22.6)	9 (22.5)	1.0
Arthralgias	3 (9.7)	3 (7.5)	1.0

[a] Pre-treatment symptoms were unknown for 5 END patients who had received prior therapy for *O. volvulus*.

Table 3. Baseline Physical Examination Findings

Physical Examination[a]	Endemic Individuals No. (%)	Temporary Residents No. (%)	*P* value
Skin manifestations			
Papules	1 (2.7)	19 (47.5)	< .001
Pigmentation changes	15 (41.7)	6 (15)	.011
Lichenification	5 (13.9)	1 (2.5)	.096
Onchocercal eye disease[b]	7 (22.6)	1 (3.3)	.053
Punctate keratitis	2 (6.5)	0 (0)	.49
Corneal scarring	4 (12.9)	1 (3.3)	.35
Subretinal scarring	1 (3.2)	0 (0)	1.0
Limb edema, unilateral[c]	1 (2.8)	5 (12.5)	.2
Lymphadenopathy	4 (11.1)	7 (17.5)	.52
Subcutaneous nodules	6 (16.7)	4 (10)	.5

[a] Baseline physical examination performed at the National Institute of Health first study visit, irrespective of prior treatment.
[b] Slit lamp examination findings were not documented in 5 END and 10 TR patients.
[c] 1 END patient had unilateral upper extremity edema. In the TR group, 4 patients had unilateral upper extremity edema, and 1 had unilateral lower extremity edema.

Table 4. Baseline Laboratory Investigations, in Patients with No History of Antifilarial Treatment Within 5 years

Laboratory Test	Endemic Individuals GM (95% CI)	Temporary Residents GM (95% CI)	*P* Value
AEC, cells/mL[a]	763.7 (432.9-1347.1)	797.5 (502.1-1266.7)	.97
Eosinophils, % [a]	13.6 (8.6-21.7)	11.8 (8.1-17.3)	.47
Eosinophilia, No. (%) [a]	13 (61.9)	17 (60.7)	1.0
WBC, x 10^3/mL [a]	5.7 (4.9-6.6)	7.3 (6.4-8.4)	.003
Leukocytosis, No. (%) [a]	2 (9.5)	4 (14.3)	.69
Polyclonal IgE, IU/mL [b]	1309 (652-2628)	251 (139-451)	< .001
Elevated polyclonal IgE, No. (%) [b]	15 (75)	11 (47.8)	.12
Polyclonal IgG, mg/dL [c]	1968 (1590-2436)	1233 (1147-1324)	.001
Elevated polyclonal IgG, No. (%) [c]	12 (80)	1 (4.5)	<.001

Abbreviations: AEC, absolute eosinophil count; CI, confidence interval; GM, geometric mean; IgE, immunoglobulin E; IgG, immunoglobulin G; WBC, white blood cell.

[a] Included 21 END and 28 TR patients
[b] Included 20 END and 23 TR patients
[c] Included 15 END and 22 TR patients

Table 5. Baseline Parasite-specific Serologic Testing, in Patients with No History of Antifilarial Treatment Within 5 Years

Parasite-specific Serology	Endemic Individuals GM (95% CI)	Temporary Residents GM (95% CI)	*P* Value
BMA-IgG, μg/mL [a]	158.1 (100-250)	175.0 (112-275)	.68
BMA-IgG Positive, No. (%) [b]	34 (94.4)	37 (92.5)	.28
BMA-IgG4, ng/mL [c]	7916 (5167-12128)	2241(1171-4290)	.005
BMA-IgG4 Positive, No. (%) [b]	31 (89)	25 (62.5)	.015
Ratio: BMA-IgG/total IgG (BMA-IgG/total IgG) [d]	0.77 (0.4-1.5)	1.7 (1.0-2.8)	.038
Ov-16 IgG4, ng/mL [e]	676 (343-1333)	618 (218-1750)	.84
Ov-16 IgG4 Positive, No. (%) [b]	18 (50)	10 (25)	.033

Abbreviations: BMA, *Brugia malayi* adult antigen; CI, confidence interval; GM, geometric mean; IgG, immunoglobulin G; Ov, *Onchocerca volvulus*

[a] Included 18 END and 22 TR patients
[b] Based on first available serum independent of prior treatment
[c] Included 15 END and 13 TR patients
[d] Included 12 END and 18 TR patients
[e] Included 11 END and 6 TR patients

FIGURE 1: Differences between Endemic Individuals and Temporary Residents in Absolute Eosinophil Count, White Blood Cell Count, and Immunoglobulin Levels

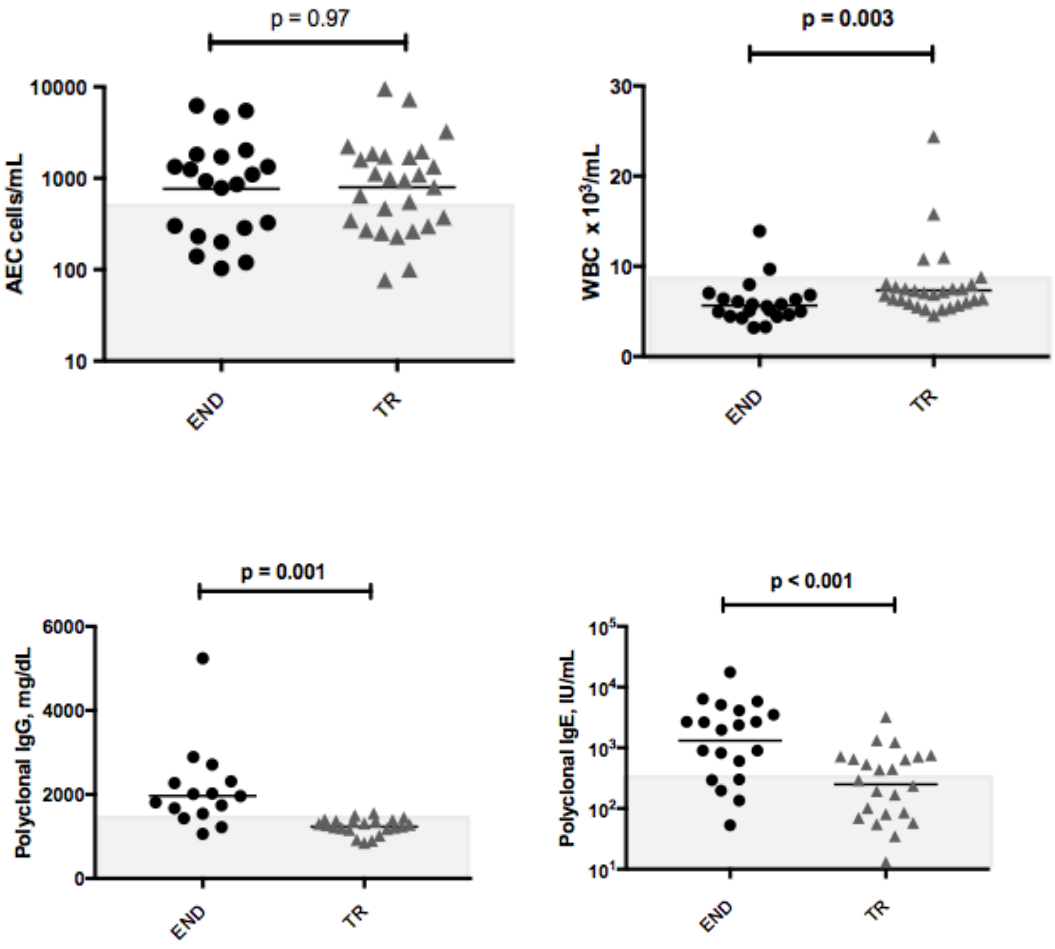

Figure 1. Horizontal lines represent the geometric mean. Data points within the shaded area lie within the normal range of the assay. Abbreviations: AEC, absolute eosinophil count; END, endemic individuals; Ig, immunoglobulin; TR, temporary residents; WBC, white blood cell count.

FIGURE 2: Differences between Endemic Individuals and Temporary Residents in Parasite-specific Serologic Testing

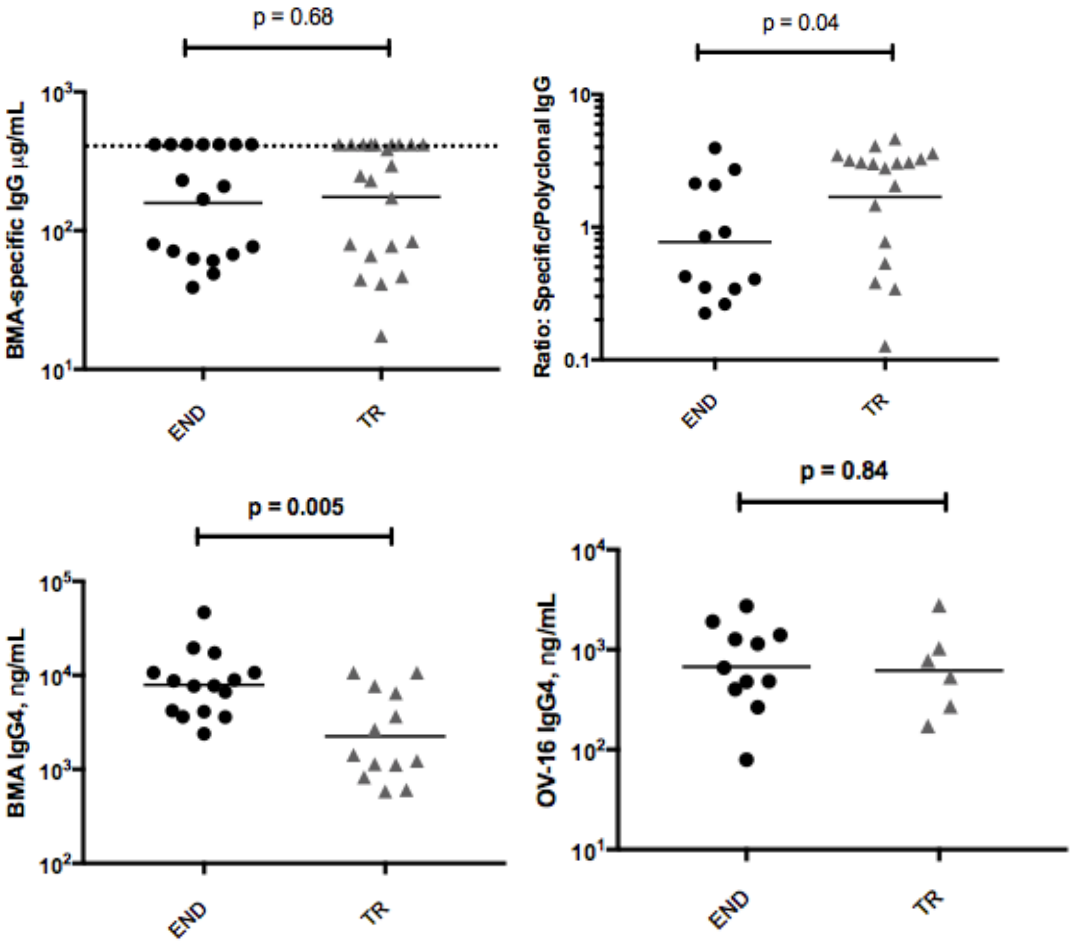

Figure 2: Horizontal lines represent the geometric mean. Dashed lines represent the upper limit of detection of the assay. Abbreviations: BMA, *Brugia malayi* adult antigen; END, endemic individuals; Ig, immunoglobulin; IgG, immunoglobulin G; OV, *Onchocerca volvulus*; TR, temporary residents.

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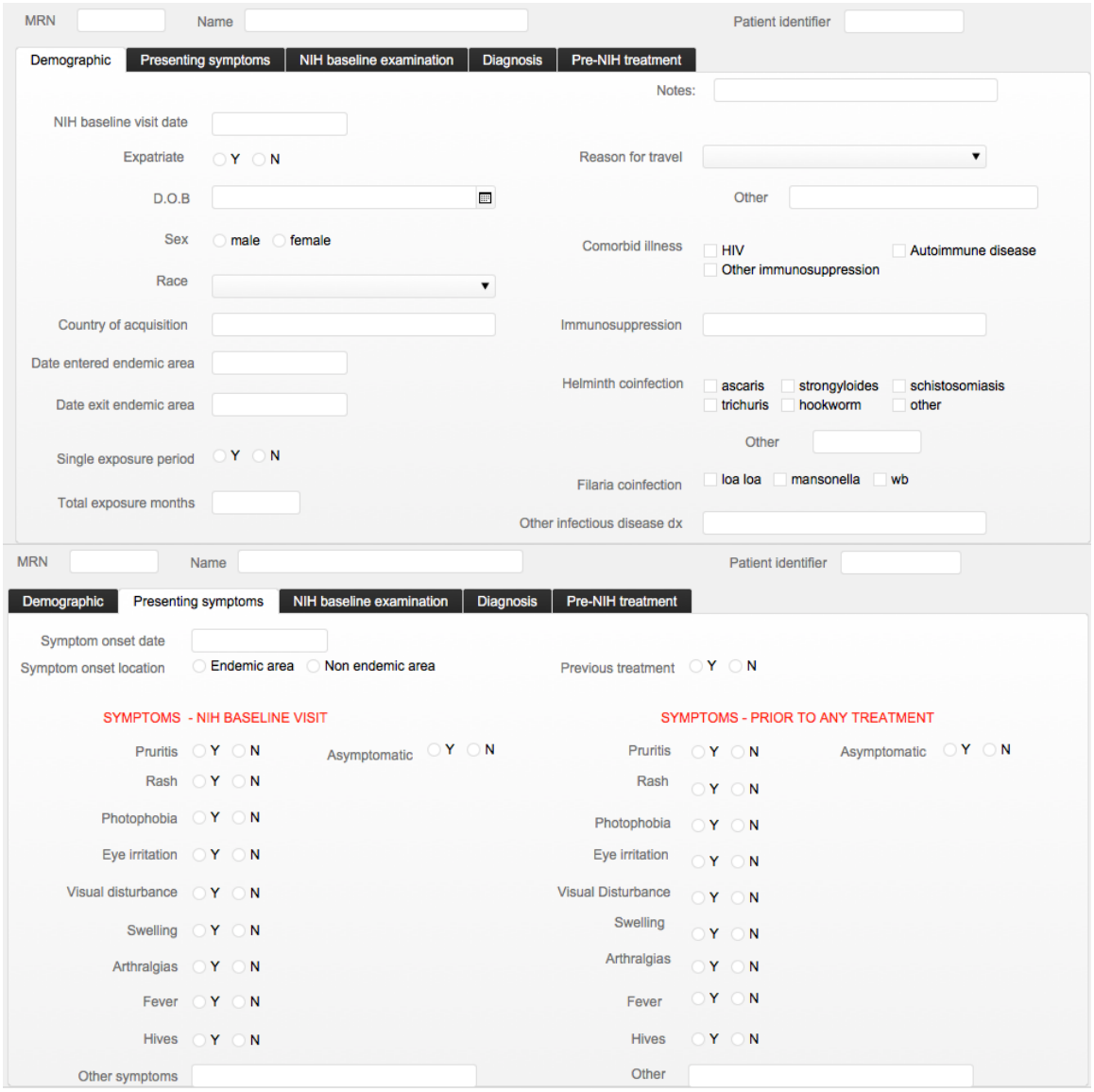
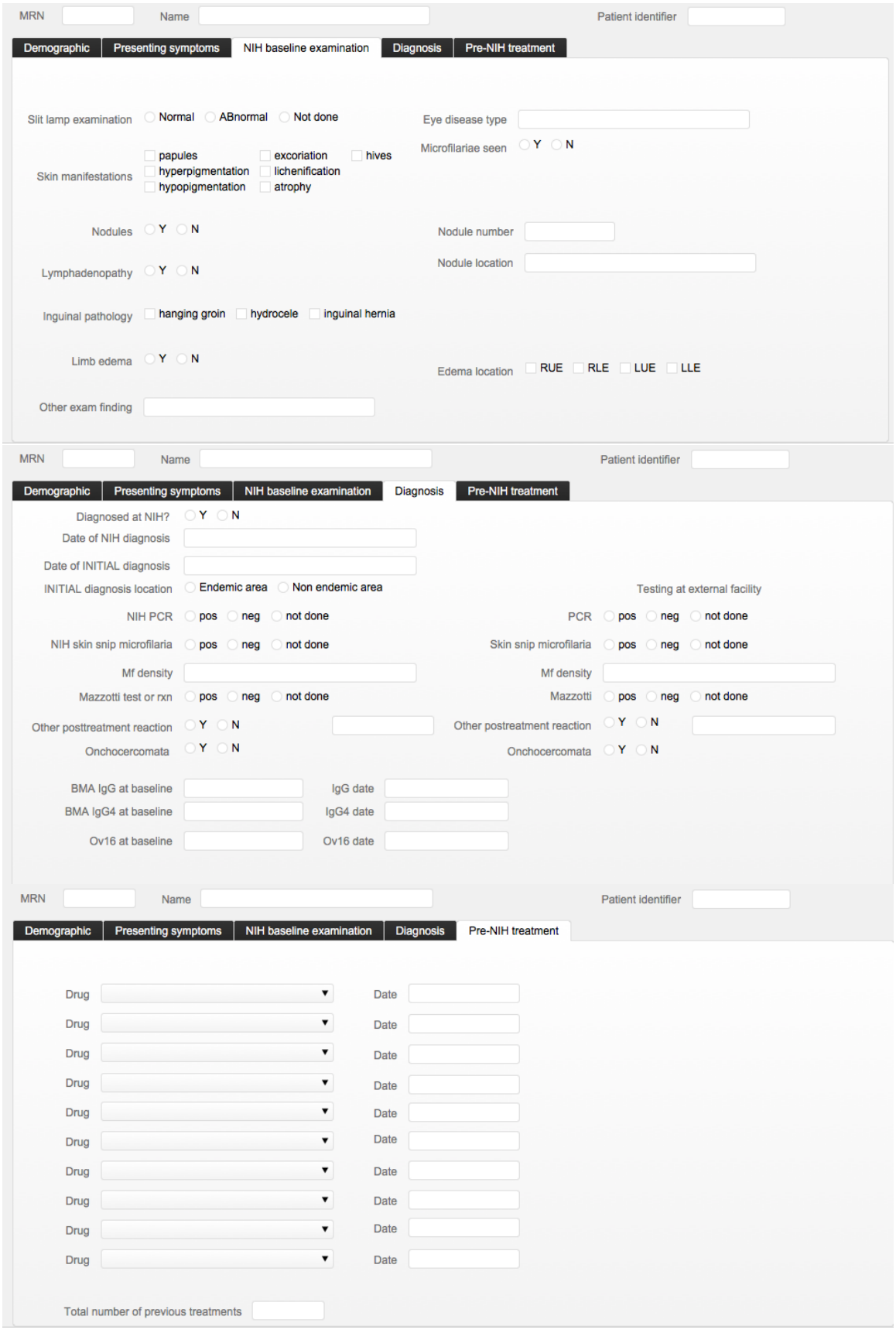
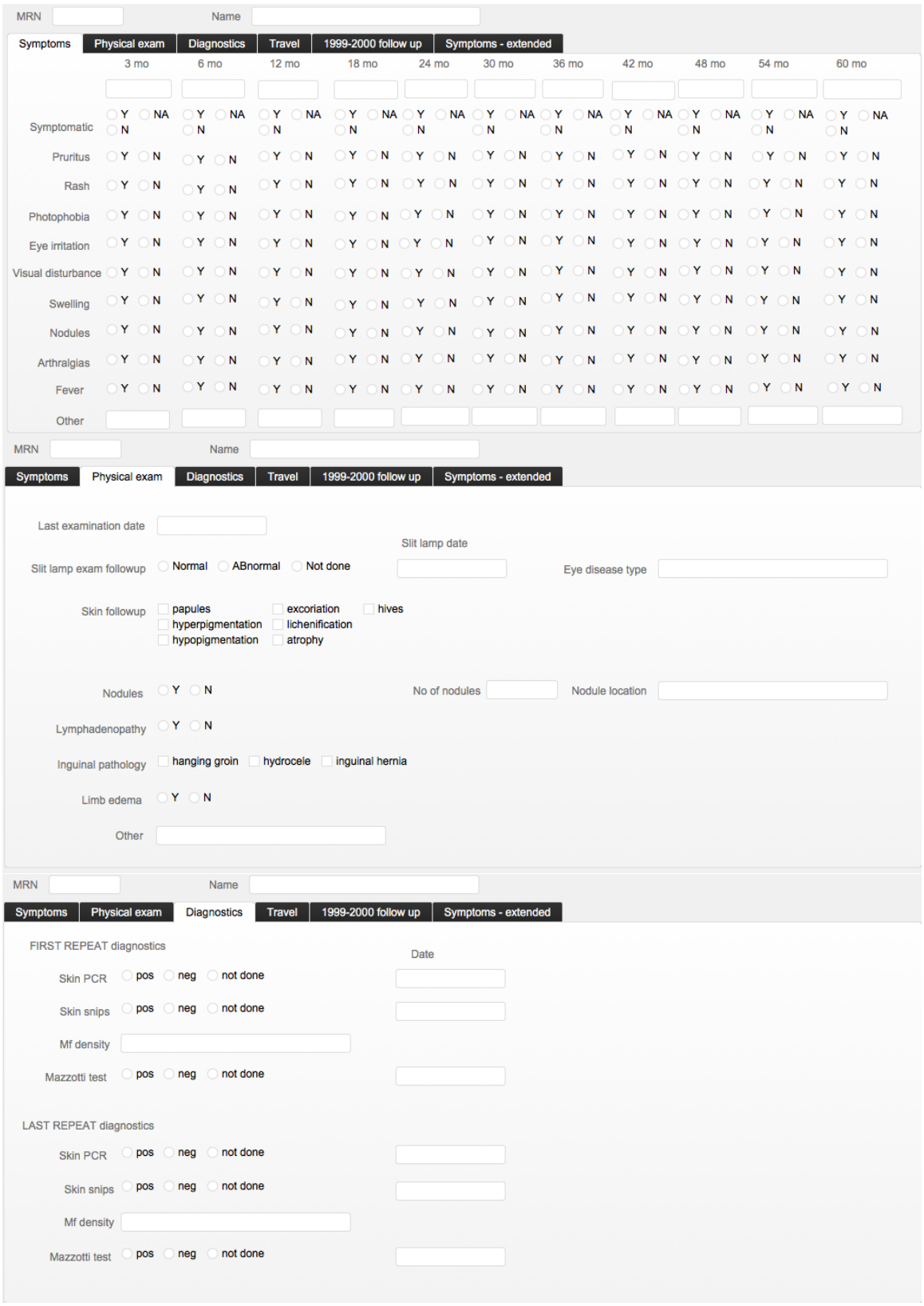
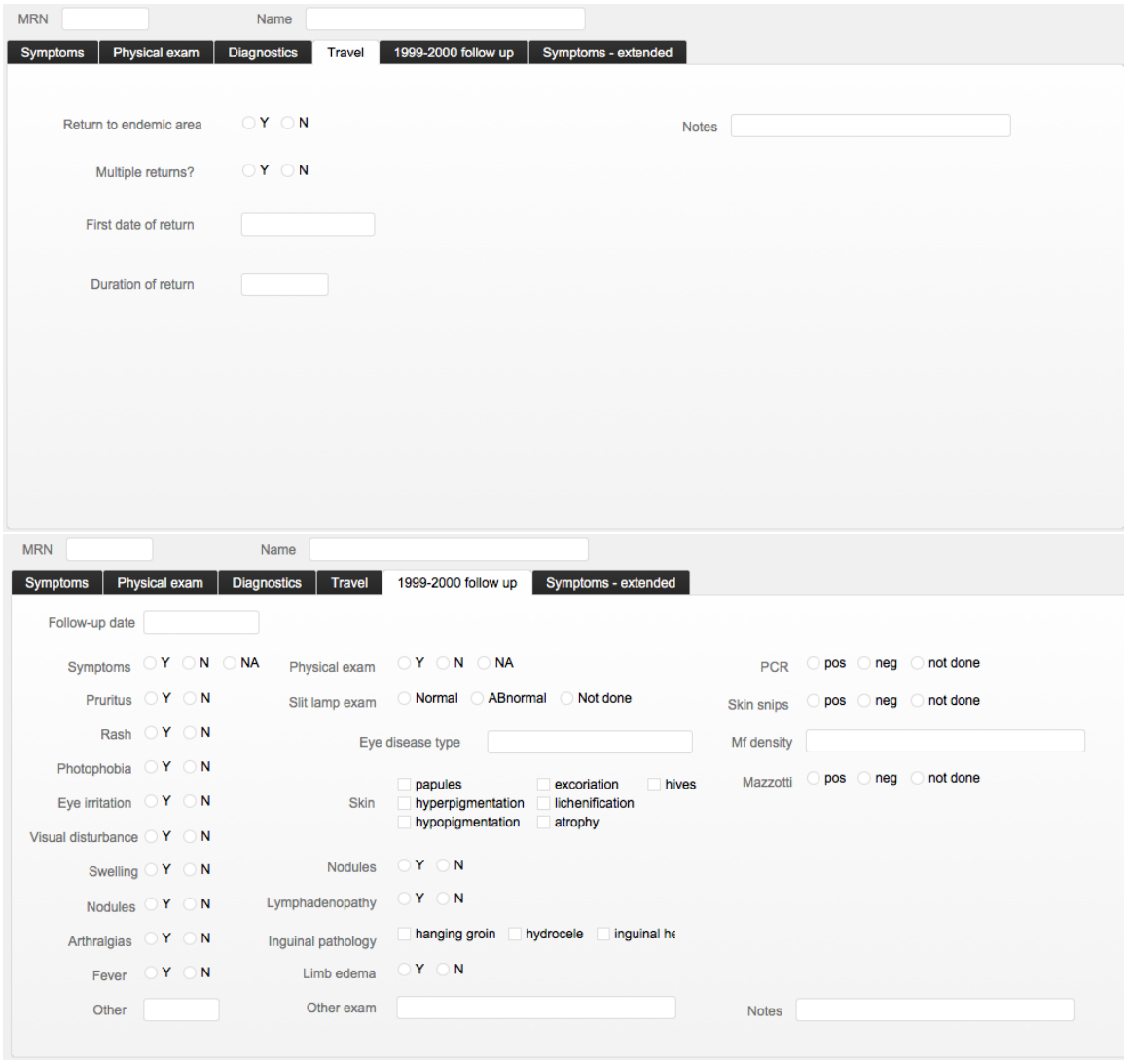
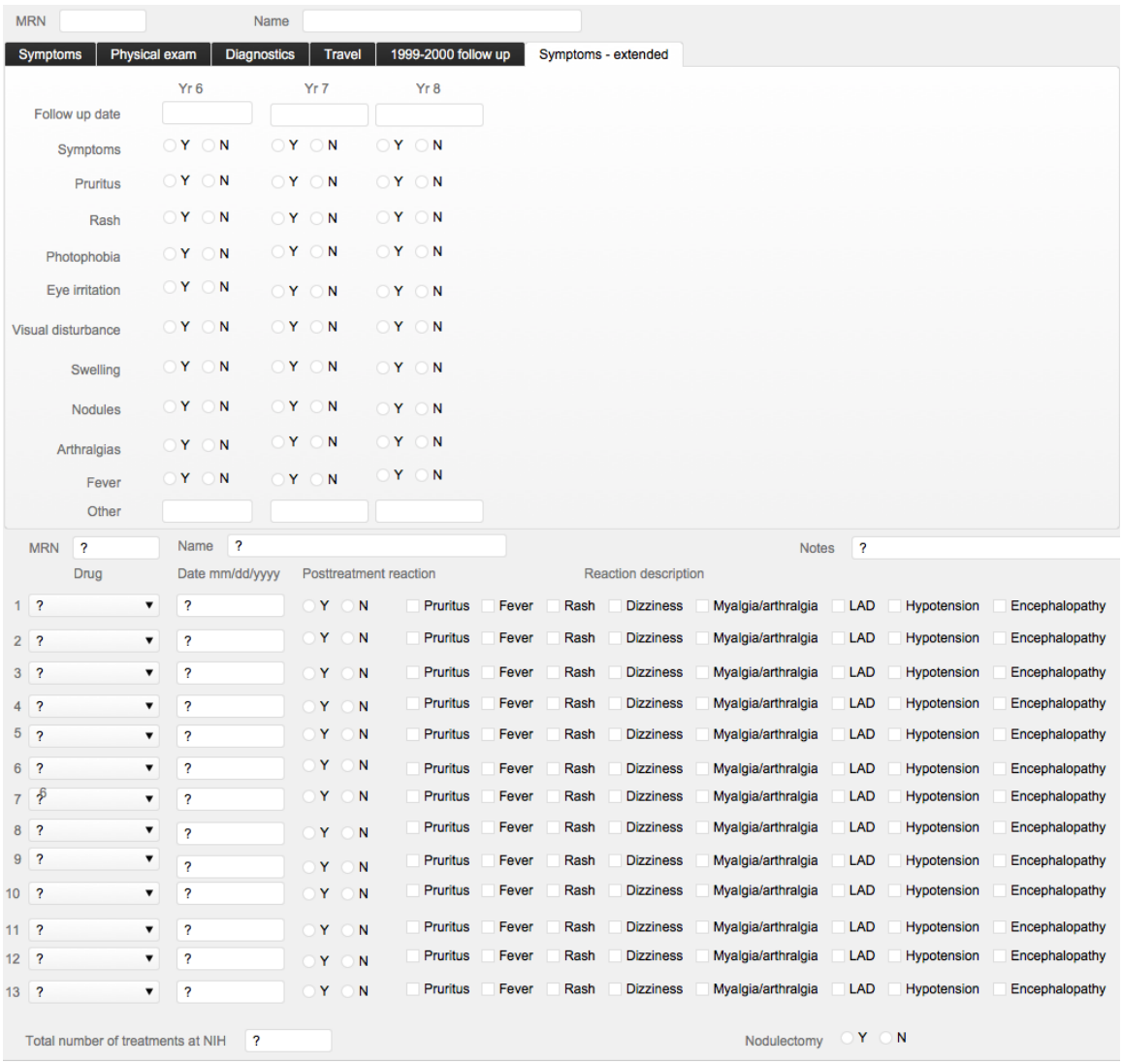
APPENDIX I: Electronic Data Abstraction Form




MRN
Name
Patient identifier
Demographic
Presenting symptoms
NIH baseline examination
Diagnosis
Pre-NIH treatment
Slit lamp examination
Normal
ABnormal
Not done
Eye disease type
Microfilariae seen
Y
N
Skin manifestations
papules
excoriation
hives
hyperpigmentation
lichenification
hypopigmentation
atrophy
Nodules
Nodule number
Nodule location
Lymphadenopathy
Inguinal pathology
hanging groin
hydrocele
inguinal hernia
Limb edema
Edema location
RUE
RLE
LUE
LLE
Other exam finding
Diagnosed at NIH?
Date of NIH diagnosis
Date of INITIAL diagnosis
INITIAL diagnosis location
Endemic area
Non endemic area
Testing at external facility
NIH PCR
pos
neg
not done
PCR
NIH skin snip microfilaria
Skin snip microfilaria
Mf density
Mazzotti test or rxn
Mazzotti
Other posttreatment reaction
Other postreatment reaction
Onchocercomata
BMA IgG at baseline
IgG date
BMA IgG4 at baseline
IgG4 date
Ov16 at baseline
Ov16 date
Drug
Date
Total number of previous treatments


MRN
Name
Symptoms
Physical exam
Diagnostics
Travel
1999-2000 follow up
Symptoms - extended
3 mo
6 mo
12 mo
18 mo
24 mo
30 mo
36 mo
42 mo
48 mo
54 mo
60 mo
Symptomatic
Pruritus
Rash
Photophobia
Eye irritation
Visual disturbance
Swelling
Nodules
Arthralgias
Fever
Other
Last examination date
Slit lamp date
Slit lamp exam followup
Normal
ABnormal
Not done
Eye disease type
Skin followup
papules
excoriation
hives
hyperpigmentation
lichenification
hypopigmentation
atrophy
Nodules
No of nodules
Nodule location
Lymphadenopathy
Inguinal pathology
hanging groin
hydrocele
inguinal hernia
Limb edema
Other
FIRST REPEAT diagnostics
Date
Skin PCR
Skin snips
Mf density
Mazzotti test
pos
neg
not done
LAST REPEAT diagnostics


MRN
Name
Symptoms
Physical exam
Diagnostics
Travel
1999-2000 follow up
Symptoms - extended
Return to endemic area
Y
N
Notes
Multiple returns?
Y
N
First date of return
Duration of return
MRN
Name
Symptoms
Physical exam
Diagnostics
Travel
1999-2000 follow up
Symptoms - extended
Follow-up date
Symptoms
Y
N
NA
Pruritus
Y
N
Rash
Y
N
Photophobia
Y
N
Eye irritation
Y
N
Visual disturbance
Y
N
Swelling
Y
N
Nodules
Y
N
Arthralgias
Y
N
Fever
Y
N
Other
Physical exam
Y
N
NA
Slit lamp exam
Normal
ABnormal
Not done
Eye disease type
Skin
papules
excoriation
hives
hyperpigmentation
lichenification
hypopigmentation
atrophy
Nodules
Y
N
Lymphadenopathy
Y
N
Inguinal pathology
hanging groin
hydrocele
inguinal he
Limb edema
Y
N
Other exam
PCR
pos
neg
not done
Skin snips
pos
neg
not done
Mf density
Mazzotti
pos
neg
not done
Notes



MRN Name

Symptoms | Physical exam | Diagnostics | Travel | 1999-2000 follow up | Symptoms - extended

	Yr 6	Yr 7	Yr 8
Follow up date			
Symptoms	Y N	Y N	Y N
Pruritus	Y N	Y N	Y N
Rash	Y N	Y N	Y N
Photophobia	Y N	Y N	Y N
Eye irritation	Y N	Y N	Y N
Visual disturbance	Y N	Y N	Y N
Swelling	Y N	Y N	Y N
Nodules	Y N	Y N	Y N
Arthralgias	Y N	Y N	Y N
Fever	Y N	Y N	Y N
Other			

MRN ? Name ? Notes ?

	Drug	Date mm/dd/yyyy	Posttreatment reaction	Reaction description
1	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
2	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
3	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
4	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
5	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
6	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
7	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
8	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
9	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
10	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
11	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
12	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy
13	?	?	Y N	Pruritus Fever Rash Dizziness Myalgia/arthralgia LAD Hypotension Encephalopathy

Total number of treatments at NIH ? Nodulectomy Y N

Curriculum Vitae
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EDUCATION

Johns Hopkins Bloomberg School of Public Health — Baltimore, MD
Thesis work: National Institute of Health, Laboratory of Parasitic Diseases — *Expected May 2017*
Master of Science, Clinical Epidemiology

University of Toronto, Faculty of Medicine — Toronto, ON
Postgraduate Residency Training, Infectious Diseases — *July 2012 – June 2014*

The Gorgas Diploma Course in Clinical Tropical Medicine — Lima, Peru
Diploma in Tropical Medicine and Hygiene — *March 2014*

University of Toronto, Faculty of Medicine — Toronto, ON
Postgraduate Residency Training, Internal Medicine — *July 2009 – June 2012*

Dalla Lana School of Public Health, University of Toronto, — Toronto, ON
Global Health Education Institute — *June 2012*
Global Health Education Initiative Program for Postgraduate Medical Trainees

University of Ottawa, Faculty of Medicine — Ottawa, ON
Doctor of Medicine — *May 2009*

Amherst College — Amherst, MA
B.A. with distinction. Majors: Neuroscience, French Lit., African Studies Certificate — *May 2005*
International Exchange Université Chiekh Anta Diop, Dakar, Senegal (2004)

ACADEMIC HONOURS

Hugo Lumbreras Medal for Overall Best Academic Performance — Lima, Peru
The Gorgas Diploma Course in Clinical Tropical Medicine 2014

Letter of Recognition for Outstanding Teaching — Toronto, ON
University of Toronto, Sunnybrook Health Sciences Centre *2012*

LICENSURE

District of Colombia Board of Medicine — *Since May 2015*
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Virginia Board of Medicine — *Since Dec 2015*
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American Society of Tropical Medicine and Hygiene *Since May 2014*

Infectious Diseases Society of America, Member *Since July 2012*

Association of Medical Microbiology and Infectious Diseases, Canada, Member *Since Oct 2013*

WORK AND VOLUNTEER EXPERIENCE

Assistant Professor, Division of Infectious Diseases and Travel Medicine *Washington*, DC
Director, Georgetown University Travel Medicine Clinic *Since Sep 2016*
Georgetown University Hospital

Special Volunteer, Laboratory of Parasitic Diseases Division Bethesda, MD
National Institute of Allergy and Infectious Diseases, National Institute of Health *Since Jan 2016*

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Toronto General Hospital Tropical Disease Unit *Since April 2015*

Courtesy Staff, Infectious Diseases Toronto, ON
St. Michael's Hospital, Toronto, Ontario *Since Mar 2015*
Trillium Health Partners, Mississauga, Ontario *Since July 2014*
Scarborough Hospital, Scarborough, Ontario *2014-2016*

Volunteer, Student Refugee Health Initiative Ottawa, ON
Performed health assessment interviews and counseling for newly arrived refugees *2006 – 2009*

Medical Volunteer, Canada-Africa Community Health Alliance Kilema, Tanzania
Provided out-patient care and assisted at in-patient rounds at Kilema Hospital *June 2007 – July 2007*

RESEARCH

PUBLICATIONS

Showler AJ, Boggild AK. *Protozoan diseases: Leishmaniasis.* IN: Quah SR and Cockerham (Eds). The International Encyclopedia of Public Health. 2nd ed. Vol 6, pp 97-102. Oxford: Academic Press 2017

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Hussein H, **Showler A**, Tan D. *Canadian "Cabin Fever": Tick-borne Relapsing Fever in Pregnancy*. CMAJ. 2014 Feb 4;186(2):131-4

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Chan W, **Showler A,** Boggild AK. *Parasitic Liver Diseases in Travelers*. IN: Zumla A, Behrens R, Memish Z, eds., Inf. Dis. Clin. North. Am., 2012 Sep; 26(3): 755–80.

Showler A, Boggild AK. *Larva Currens Presenting 38-Years after Presumed Exposure*. Journal of Cutaneous Medicine and Surgery 2012; 16(6):433-435.

POSTERS

Kopalakrishnan S, Macrae C, Klowak M, **Showler A**, Klowak S, Boggild AK. Evaluation of Safety Tool for Ambulatory Leprosy Patients at Risk of Adverse Outcome. Poster presented at: American Society of Tropical Medicine and Hygiene Annual Meeting; 2016 Nov 13-17, 2016; Atlanta, GA.

Yeung S, Mourad O, Klowak M, **Showler A**, Klowak S, Boggild AK. Implementation and Evaluation of a Quality and Safety Tool for Ambulatory Strongyloidiasis Patients at High Risk of Adverse Outcome. Poster presented at: American Society of Tropical Medicine and Hygiene Annual Meeting; 2016 Nov 13-17, 2016; Atlanta, GA.

Tutert M, Kariyawasam R, Lau R, **Showler A**, et al. *Surveillance for Molecular Markers of Drug Resistance in Plasmodium falciparum Imported to Ontario*. Poster presented at ASTMH annual meeting 2015; 2015 Oct 25-29; Philadelphia, PA.

Showler A, Burry L, Bai A, et al. *A Normal Transthoracic Echocardiogram rules out Infective Endocarditis in Low-risk Patients with Staphylococcus aureus Bacteremia*. Poster presented at: ID week 2014; 2014 Oct 8-12, 2014; Philadelphia, PA.

Science S, **Showler A**, Burry L, et al. Staphylococcus aureus Bacteremia in Children: A Retrospective Review at a Tertiary Care Hospital. Poster presented at: ID Week 2014; 2014 Oct 8-12, 2014; Philadelphia, PA.

Thampi N, **Showler A**, Burry L, et al. *Cost-of-Illness Analysis of Staphylococcus aureus Bacteremia*. Poster presented at: ID Week 2013; 2013 Oct 2 – 6; San Franciso, CA.

Low C, **Showler A**, Mcintyre S, et al. *Transmission of Brucella Meletensis to a Healthcare Worker Outside of the Microbiology Laboratory*. Poster presented at: AMMI Canada – CACMID Annual Conference; 2013 April 4 – 6; Quebec City, Canada. **Recipient of CCM 2013 Dr. Kenneth Rozee Memorial Poster Award.**

Hussein H, **Showler A**, Tan D. *Canadian "Cabin Fever": Tick-borne Relapsing Fever in Pregnancy*. Poster presented at: AMMI Canada – CACMID Annual Conference; 2013 April 4 – 6; Quebec City, Canada

EDUCATIONAL ACTIVITIES

PRESENTATIONS

Showler A, Nutman T. (2016). Differences in the Clinical and Laboratory Features of Onchocerciasis in Endemic and Non-endemic Populations. Oral abstract presented at: American Society of Tropical Medicine and Hygiene Annual Meeting; 2016 Nov 13-17, 2016; Atlanta, GA.

Boggild A, Caumes E, Connor BA, Chakrabarti S, Parola P, Hynes N, Keystone J, Libman M, Nash T, Schwartz E, **Showler AJ**, Hamer D, Kain K. (2016). Cutaneous and Mucocutaneous Leishmaniasis in International Travelers: Results from the GeoSentinel Surveillance Network. Presented at: American Society of Tropical Medicine and Hygiene Annual Meeting; 2016 Nov 13-17, 2016; Atlanta, GA.

Medical Grand Rounds, Toronto City-Wide Toronto, ON
Title: *Staphylococcus aureus* bacteremia: why do we accept a quarter of these patients dying? Jan, 2015

Medical Grand Rounds, Toronto City-Wide Toronto, ON
Title: Ebola Outbreak 2014 *Aug 2014*

Infectious Disease Toronto City-Wide Grand Rounds Toronto ON
Title: Ebola Outbreak 2014 *May 2014*

TEACHING ACTIVITIES

Teaching Assistant, Emerging Infections – Bloomberg School of Public Health **Baltimore**, MD
Presented seminar on MERS-CoV, assisted with course article selection *April-May 2015*

Microbiology seminar leader – University of Toronto Toronto, ON
Led case-based bacteriology and mycology seminars for 2nd year medical students *Sep 2013*

Postgraduate resident small-group teaching – University of Toronto Toronto, ON
Taught residents and medical students about core ID topics – total 125 hours *July 2012 – June 2013*

Postgraduate medical lecturer, PGY1 Academic half-day – University of Toronto Toronto, ON
Presented an approach to opportunistic infections in the Emergency Department *Nov 2012*